Collaboration Study Proposal

Introduction:

Currently, *E. coli* is the most commonly used chassis in synthetic biology. While it is an excellent cell factory, it is by no means omnipotent. For example, it uses expensive feedstocks like sugars as substrates, it often produces CO_2 as the main waste which burdens the environment, and its metabolic potential only represents one drop in the vast ocean of diverse microbial metabolisms. Therefore, it is of great interest to develop alternative hosts for synthetic biology. Our team believes the utilization of methanogenic archaea or methanogens can serve as a promising alternative host in many areas where *E. coli* is less desirable. For example, many methanogens are autotrophs and nitrogen fixers, which enable them to use CO_2 and N_2, arguably the simplest and cheapest carbon and nitrogen sources, to make high value biochemicals. Methanogens also catalyze the last step in anaerobic digestion where raw organic waste such as manure and garbage are converted to biogas consisted of mainly the fuel methane. Last but not the least, archaea may hold great potential to help fight human and animal diseases due to the high similarities in information processing systems between archaea and mammals.

However, few iGEM teams are working on methanogens or archaea. We believe this is not because methanogens are any less important or useful than *E. coli* for synthetic biology but rather due to the shortage of tools and parts available to the iGEM community. As one of the first iGEM teams working on archaea, we have established a few tools and parts for *Methanococcus maripaludis*, a model organism for methanogens. We have demonstrated the feasibility of biochemical production and gene expression monitoring. To raise awareness of and get more teams involved in archaeal studies, we invite you to collaborate with us on an experiment to test the ribosome binding site (RBS) specificity in *Methanococcus*.

Description of our study:

Because *Methanococcus* exhibits a natural blue-green autofluorescence, we are using mCherry as a marker instead of GFP. In short, the transformed cells are first grown to a specific absorbance, and the cells are harvested, lysed and resuspended in PIPES buffer, releasing mCherry proteins in the cell extracts. The cell extracts are then subjected to oxygen exposure to activate the fluorophore before measuring fluorescence. All this is done at the University of Georgia iGEM lab, and we have generated cell extracts that are currently stored in the freezer and can be shipped to fellow iGEM teams.

At this point, we have already constructed an mCherry library in *M. maripaludis* which carries over 30 point mutations in the ribosomal binding site (RBS), and we are currently measuring the fluorescence from each mutation. We recognize that few iGEM teams possess anaerobic chambers and the ability to make anaerobic medium suitable for growth of *Methanococcus maripaludis.* Therefore, for the collaboration effort, we only requiring participating iGEM teams to measure fluorescence of mCherry proteins, which does not require anaerobic chambers or media. Inspired by the Interlab that iGEM headquarters initiated last year, we would like to distribute extracts of *M. maripaludis* containing our mCherry mutants to fellow iGEM teams for them to measure the protein fluorescence originated from each mutation.

Registration & Timeline:

There will be no deadline for registration. Please register by completing the linked form: (http://goo.gl/forms/2F3Yb0ABrE).

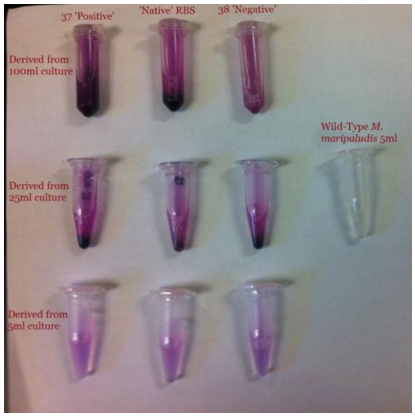
Collaboration Study Requirements:

Our requirements are very simple to follow. Within our study we plan to adhere to similar rules as presented on the iGEM *E. coli* InterLab Study web page such as that of the devices and protocol forms. However, instead of requiring cloning, transformation and fluorescence measurement of GFP, as the InterLab *E. coli* Study recommends, our collaboration design requires only the fluorescence measurement for the mCherry library, which is the last step of our provided protocol. To take fluorescence readings, we recommend using Gen5 software and a BioTek Synergy HT plate reader with the filter set to 590/20 nm for excitation and 645/40 nm for emmission to measure 2µL of the provided extract, suspended in 198µL of 25mM PIPES buffer at pH 6.8. However, any alternative measurement methods are welcome, as long as protocols are fully described.

Our data collection will be simple with the completion of the Google spreadsheet titled "2015 Archaea Collaboration Datasheet [Insert Team Name Here]." As noted the team name will be inserted into the title and shared to both stevenkodish@gmail.com and akshaychandora@gmail.com. The simplicity of this study will optimize data collaboration between numerous teams. After proper compilation, data will be made available to all participating teams for us to assess the reproducibility of measurements among teams.

Required Devices:

Pictured below from left to right are the three mCherry devices we developed last year, BBa_K1383001, BBa_K1383000, and the BBa_K1383002 that will be used as the minimum requirement in our InterLab Study. This will establish a baseline for mCherry measurement. Our study will further seek to compile the fluorescence measurements of about 100 *M. maripaludis* colonies in the mCherry library we created this year, which would cover ~30 possible variants having single mutations in the RBS, spacer, and start codon region.



Chassis:

The chassis being used is *Methanococcus maripaludis*, strain S0001.